Ornis Fennica 73:131–136. 1996

Increased eggshell porosity in replacement clutches of the Black-headed Gull *Larus ridibundus*

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Received 18 December 1995, accepted 7 February 1996

Water vapour permeability, pore density and thickness of the eggshells from initial and replacement clutches of the Black-headed Gull (*Larus ridibundus*) were measured and compared. The permeability of the eggshells of replacement clutches was, on average, higher than that of either the corresponding initial clutches or the control sample of late initial clutches initiated during the same period as replacement clutches. Thus, the difference cannot be explained merely on the basis of any seasonal trend. The difference was caused by the higher number of pores and not by the thinner shells of the replacement eggs. Eggshell porosity increased during the first days of incubation. Changes in eggshell porosity can probably serve as an early signal of changing environmental properties affecting eggshell quality.

1. Introduction

Eggshell properties have been studied by ecologists since Ratcliffe's (1967) work in which a decrease in eggshell weight due to environmental contamination was demonstrated in some raptors. Recently, the negative influence of atmospheric acid precipitation and heavy metal contamination on the calcium metabolism of birds and on eggshell quality has been identified (Drent & Woldendorp 1989, Mitchell 1989, Graveland 1990, Graveland et al. 1994). Both eggshell thickness and porosity may be affected by environmental factors causing changes in the functional properties of the shell and leading to egg desiccation and lowered hatchability (Graveland et al. 1994, Eeva & Lehikoinen 1995). In this context, a better knowledge of the sources and correlates of the between-clutch variation in the functional traits of the eggshell, such as water vapour permeability which depends both on porosity (or pore density) and shell thickness, is of great importance. Most of the related studies, however, have dealt with a variation in the thickness and/or relative weight of the eggshell (e.g. Eeva & Lehikoinen 1995), while investigations where shell porosity and/or permeability to gases in wild birds were estimated directly are almost lacking.

I report here the results of an experimental study on the variation of some functional traits of the eggshell of the Black-headed Gull. I will also demonstrate an increase in eggshell porosity and permeability to water vapour in replacement clutches compared with early and late initial clutches.

2. Material and methods

The study was carried out in two adjacent colonies of the Black-headed Gull located on small sea islets in Rame Bay, Läänemaa, Estonia (58°34´N, 23°38´E). Nests and eggs were individually marked using a felt-tip pen. Thirty randomly chosen complete three-egg clutches with known time of laying and intra-clutch sequence were removed from nests 1–7 (average 4.4) days after the onset of incubation. The eggs were photographed. A graphics digitizer was used for the input of egg contour data from the photographs; a special program (for the description of an earlier version, see Mänd et al. 1986) was applied for smoothing the data and for estimating eggshell area and volume. Note that these parameters were calculated from the egg contour using trapezoidal integration instead of deriving them from linear measurements. Thus, individual differences in the shape did not influence the accuracy of volume estimation.

The eggs were placed in the thermostatically controlled desiccator with a surplus of silica gel at 25°C. After five hours the eggs were weighed on an analytical balance with an accuracy of 0.01 g, returned to the desiccator and maintained there for approximately eight days. Such conditions do not permit the development of the avian embryo and keep the water vapour pressure difference across the eggshell nearly constant (Ar et al. 1974). After this period the eggs were re-weighed. Water vapour conductances of the eggs were calculated on the basis of their weight loss (for details, see Ar et al. 1974). In further analyses, I used water vapour permeability as an area-specific measure instead of water vapour conductance, since the former has been stated to be more useful in comparing differences within a species (Ar et al. 1974, Carey et al. 1983).

Subsequently, the eggs were emptied and the eggshell was broken into fragments. The number of pores per unit area of the shell (pore density) was estimated according to the method used by Tyler (1953) and Tullett (1975). The shell fragments were boiled in 2.5% NaOH to remove protein fibres. They were then briefly etched in concentrated nitric acid to enlarge pore channels, after which they were washed in distilled water and dried. Pores were counted in transparent light, using a projection apparatus. The mean number of pores of twenty randomly selected 5-mm^2 areas around the blunt end of each egg was used as a measure of porosity.

Eggshell thickness (without membranes) was measured using a micrometer calliper at five points selected randomly around the equator, since variation in eggshell thickness of a single egg has been shown to be lower around the latitudes than longitudinally (Tyler 1961). The mean of all five measurements was taken as eggshell thickness.

Twenty pairs of gulls were checked for laying replacement clutches after removing the initial ones. Fourteen of them laid replacement clutches in an interval of 3–9 (average 6.9) days after the loss of initial clutches. All these clutches, as well as a similar-sized sample of late initial clutches which were laid during the same period of time as the replacement clutches, were removed and subjected to analysis. Note that the incubation stage of all three samples fell into the same interval of 1–7 days with the respective means of 4.4 (early initial clutches), 3.6 (replacement) and 4.9 (late initial) days.

3. Results

3.1. The effect of incubation

Eighty-five eggs (74 with pores counted) from three-egg initial clutches with incubation stages from one to seven days were analyzed in order to reveal the effect of incubation on the studied traits. Both the pore number and water vapour permeability tended to increase with the incubation stage from the first to the seventh day of incubation (permeability: beta = 0.503, $F_{1,83}$ = 28.060, $P < 0.001$; pore number: beta = 0.484, $F_{1,72}$ = 22.014, $P < 0.001$). No significant variation due to the incubation stage was detected in shell thickness during this period (beta = 0.178, $F_{1,83}$ = 2.728, $P = 0.102$). Prior to further analysis, all measured values of eggshell traits were corrected to the 0th day of incubation using linear regressions derived from empirical data.

3.2. The effect of laying sequence

The position of an egg in the laying order has been shown to be correlated with some egg parameters in Laridae (see references in Ojanen 1983). Since no data on the effect of laying order on eggshell porosity in Black-headed Gull were available, I checked it on the same sample of clutches mentioned above, after correction for incubation stage. The laying order had no significant effect on total variation in any of the parameters studied (ANOVA; permeability: $F_{2,82}$ = 1.589, $P = 0.210$; pore density: $F_{2,71}$ = 0.769, $P = 0.467$; thickness: $F_{2,82}$ = 1.044, $P = 0.357$; volume: $F_{2,82}$ = 0.750, $P = 0.476$). These results are of importance in the context of further analysis because some of the replacement nests contained fewer than three eggs.

3.3. Individual differences

There were significant differences between initial clutches of different females in shell permeability ($r^2 = 0.533$, $F_{28,56} = 2.286$, $P = 0.004$), pore density ($r^2 = 0.628$, $F_{24,49} = 3.448$, $P < 0.001$), shell thickness ($r^2 = 0.523$, $F_{28,56} = 2.193$, $P = 0.006$) and volume ($r^2 = 0.825$, $F_{28,56} = 9.454$, $P < 0.001$). Only the clutch means or the single (first) eggs of the clutches were used in further analyses to avoid pseudoreplication.

3.4. Early initial, replacement and late initial clutches

Table 1 gives the mean values of permeability, pore density, thickness and outer volume of the eggshell in initial, replacement and control samples of late initial clutches initiated during the same time interval as the replacement clutches.

Both the eggshell permeability and pore density of the replacement clutches were significantly higher than those of the initial clutches. There was no significant difference between the eggs of earlier and late samples of the initial clutches. Eggshell thickness did not vary significantly in different samples. The same held true when only one egg per clutch (the first one) was subjected to the analysis.

Egg volume in both the replacement and late initial clutches tended to be smaller than in the earlier initial clutches, but the difference was statistically significant only in the case of late initial clutches. When only first eggs per clutch were used in calculations, a significant difference was revealed also between replacement and initial clutches ($t_{26} = 2.098$, $P = 0.046$).

When the clutch means of the corresponding initial and replacement nests were compared by the means of the paired t-test, a significant difference was again revealed in the case of shell permeability ($t_{13} = 3.089$, $P = 0.009$) and pore density ($t_{12} = 2.209$, $P = 0.047$), but not in shell thickness ($t_{13} = 0.090$, $P = 0.929$) and volume ($t_{12} = 1.705$, $P = 0.114$).

However, there was no significant correlation between the eggshell parameters of the corresponding pairs of the initial and the replacement clutches ($n = 14$; permeability: $r = -0.173$, $P = 0.555$; pore density: $r = -0.229$, $P = 0.431$; shell thickness: $r = -0.013$, $P = 0.966$; egg volume: $r = 0.265$, $P = 0.359$).

4. Discussion

4.1 The effect of incubation

Water vapour permeability of the eggshell tended to increase during the first days of incubation in the Black-headed Gull. This result agrees with the findings of Carey 1979, Hanka et al. 1979 and Kern 1986, who demonstrated that the water vapour conductance of the eggshell in a number of species increased in the first days of incubation. The exact mechanism responsible for this phenomenon is still unclear. Since the eggshell thinning due to the reabsorption of shell calcium by the developing

Table 1. Eggshell water vapour permeability (K_{H_2O}), pore density, thickness and volume in early initial, replacement and late initial nests (mean ± S.E.). n = 14 clutches (only the clutch means are used for calculations); P denotes the significance level of the difference from initial clutches (two-tailed t-test). Shell permeability is given in cm^3 (STP) s^{-1} cm^{-2} $torr^{-1}$ 10^6.

Eggshell parameter	Early initial	Clutch types Replacement	Late initial
K_{H20}	1.61 ± 0.069	1.97 ± 0.073 P = 0.002	1.63 ± 0.096 P = 0.879
Pores mm^{-2}	1.51 ± 0.264	2.81 ± 0.329 P = 0.005	1.65 ± 0.232 P = 0.698
Shell thickness (µm)	195.8 ± 1.77	196.1 ± 3.67 P = 0.933	193.5 ± 2.06 P = 0.399
Volume (cm^3)	38.0 ± 0.87	36.1 ± 0.81 P = 0.116	34.4 ± 0.61 P = 0.002

embryo has been shown to take place mainly during the second half of the incubation period (Simkiss 1961), there should be another reason for increasing shell permeability in the first half of incubation. Goryainova and Tarnovskaya (1975) and Booth (1989) showed that the number of open pores increased during incubation in eiders, swans and domestic fowl. On the contrary, Kern et al. (1992) found no increase in pore numbers during incubation in the Pied Flycatcher. Nevertheless, there is some evidence that pores enlarge during incubation (Kern et al. 1992). This may be due to the erosional effect of enzymes or acids which diffuse into the pores (Simkiss 1980, Kern et al. 1992). In the Black-headed Gull the number of pores which became visible after treating the eggshell with NaOH and nitric acid increased significantly even during the first seven days of incubation. Some erosion may start already in the early period of the incubation, increasing the number of large, detectable pores. Irrespective of the exact cause of this finding, it is clear that functional traits of the eggshell should be corrected considering this effect of incubation before further analyses.

4.2. Difference between initial and replacement eggs

The shell of the replacement eggs of the Black-headed Gull contained, on average, more pores and was more permeable to water vapour than the shell of the corresponding initial eggs. At the same time, the average shell thickness in the two groups compared did not differ significantly from each other (Table 1). Note that the water vapour permeability and pore density of the shells were estimated by completely different and independent methods, hence the possibility of an artefact caused by some unknown methodological cause seems unlikely.

In the case of some other traits (e.g. egg and clutch sizes) differences between initial and replacement clutches have usually been attributed to a common seasonal trend occurring also among initial clutches (e.g. Feare 1976, Birkhead & Nettleship 1984, Verhulst & Tinbergen 1991). In this study, too, egg volume in both the replacement and late initial clutches tended to be smaller as compared with the initial clutches. Indeed, the size of the replacement eggs may have simply followed a seasonal trend. This was not so in the case of eggshell permeability and the number of pores in the shell. The eggshells of the early and late samples of the initial clutches did not differ from each other in pore density or permeability to water vapour (Table 1).

No significant difference was detected in the shell thickness of the groups compared. I conclude therefore that the increased permeability of the eggshell in the replacement clutches of the Black-headed Gull was caused by higher porosity rather than by shell thinning. This is in good accordance with earlier findings that both intraspecific and interspecific differences in the water vapour conductance of eggs result from differences in the functional pore area rather than from eggshell thickness (Sotherland et al. 1980, Carey et al. 1983, 1987, 1989ab, Whittow & Grant 1985, Whittow et al. 1985). Differences in the functional pore area are, in turn, caused by the variation in the number of pores rather than by their size (Ar & Rahn 1985, Whittow & Grant 1985, Carey et al. 1987, 1989ab, Carey 1990), both between and within species. Eggshell porosity, thus, seems to be phenotypically a more plastic trait than shell thickness.

No data were available to guess a proximate cause of the higher porosity of the eggshell in replacement clutches. Calcium deficit is an unlikely reason since there is always a surplus of calcium-rich material around the breeding colony of seagulls. Debout and Hempleman (1994) have recently shown that calcium deficient diets in domestic hens caused eggshell thinning, but had little effect on total functional pore area. Therefore, certain physiological constraints seem to be a more likely explanation than Ca-deficiency. Unfortunately, the pore formation mechanism, as well as the cause of the variation in eggshell porosity and the mechanism and control of the mobilisation of the shell calcium, are not fully understood (Carey 1983, Packard 1994).

In order to elucidate the possible mechanism, corresponding pairs of initial and replacement clutches belonging to the same females were compared (last paragraph of Results). If calcium deficiency (due to changed feeding behaviour of the birds or the female's ability to mobilize its body reserves) was the case, one would expect that birds which already had trouble allocating enough calcium to the eggshell in the first clutch would have had even more trouble allocating it into the replacement eggs. In this case, a positive correlation between the porosity/permeability of the eggshells of corresponding pairs of clutches would have been expected. In addition, the more porous the eggshell was in the initial clutch, the bigger the difference in eggshell porosity/permeability between these pairs of clutches would be. Alternatively, if a trade-off existed between the amounts of calcium allocated to the first and replacement eggs, one would expect a negative relationship between the shell porosity estimates of the initial and replacement eggs of the same female. In fact, there was no significant correlation between the corresponding pairs of clutches. There was also no relationship between the eggshell porosity in the initial clutches and the interclutch interval. A possible explanation is that certain physiological constraints indeed exist, and the traits of the eggshell of the replacement eggs fall, on average, closer to the lower limit of the reaction norm than those in the initial eggs.

It is not fully excluded that an increase in the porosity of replacement eggs represents an adaptation compensating for some unavoidable poor properties of such clutches (small clutch and egg size, late hatching etc.). In fact, although high permeability results in greater water loss, it also allows larger amounts of respiratory gas exchange across the shell. Perhaps higher permeability allows accelerated development of the embryo (Burton & Tullett 1983, Burton 1987). The probability of adaptive explanation depends on how the 1.22-fold increase in water loss (Table 1) in the replacement eggs affects the embryo or the subsequent life history of the young. The results of studies on wild birds (e.g., Carey et al. 1983, 1989a, Carey 1994) suggest that eggshell conductances in conspecific populations tend to be adapted to the varying conditions of barometric pressure and the ambient O_2 level. This, together with the fact that the mortality of chicken embryos rises if the rate of daily water loss is either increased or decreased by variation of incubator humidity (Ar & Rahn 1980), has led to the recognition of the vital importance of the restricted limits of eggshell permeability to water vapour. Other studies (Simkiss 1980, Carey 1986), however, have demonstrated a high tolerance of embryos to even a substantial variation in water loss. Therefore, it is hard to say which could be the actual consequences of an increase in eggshell permeability in the replacement eggs of the Black-headed Gull.

An important message arising from this study is that eggshell porosity seems to be a phenotypically more plastic trait than shell thickness. Changes in porosity can probably also serve as an early signal of changing environmental properties affecting eggshell quality. Thus, variation in eggshell porosity deserves more attention in respective studies than it has so far attracted.

Acknowledgements. I am grateful to Endla Pruks for assistance in laboratory analysis and to Cynthia Carey, Mikko Mönkkönen and three anonymous referees for helpful comments on the earlier versions of this manuscript. Michael Stubbe and University of Halle are acknowledged for the financial support for improving the computer program for egg morphology analysis. The study was partly supported from grant No. 872 by the Estonian Science Foundation.

Selostus: **Lisääntynyt munan kuoren huokoisuus naurulokin uusintapesyeissä**

Kirjoittaja vertasi naurulokin munan kuoren paksuutta, huokosten määrää ja vesihöyryn läpäisevyyttä aikaisin ja myöhään aloitetuissa ensimmäisissä pesyeissä sekä uusintapesyeissä Eestissä. Uusintapesyeissa munan kuoren läpäisevyys oli korkeampi kuin ensimmäisissä pesyeissä. Ero oli havaittavissa myös uusintapesyeiden ja samaan aikaan aloitettujen ensimmäisten pesyeiden välillä. Ero johtui munan kuoren huokosten suuremmasta määrästä uusintapesyeiden munissa ei kuoren paksuuden eroista. Huokosten määrä kasvoi haudonnan alussa. Koska huokosten määrä on fenotyyppisesti joustavampi ominaisuus kuin kuoren paksuus, huokoisuus mahdollisesti voi toimia varhaisena merkkinä ympäristömuutoksista. Huokoisuuden tarkkaa merkitystä haudonnan aikaiseen kuolleisuuteen ei kuitenkaan tunneta.

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